Highlights from other journals – October 2000

SH2-Directed ligands of tyrosine kinase

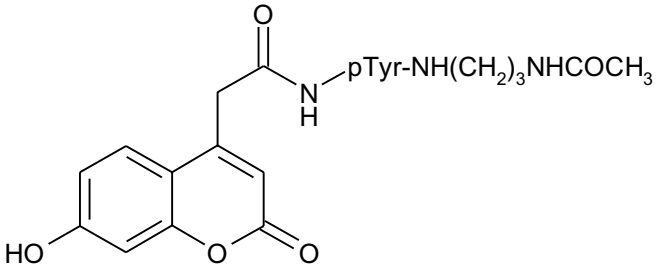
Tyrosine-specific protein kinases are composed of two subfamilies: Receptor tyrosine kinases, which are integral membrane proteins, and nonreceptor cytoplasmic counterparts. The former, upon co-ordination to specific extracellular ligands, forms aggregates and subsequently suffers phosphorylation of key tyrosine residues. Cytoplasmic signalling proteins, including nonreceptor tyrosine kinases, co-ordinate to these phosphotyrosine (pTyr) residues through Src homology 2 (SH2) domains. This binding event triggers the activation of specific intracellular signalling pathways, ultimately leading to a cellular response in reaction to the extracellular stimulus. SH2 domains play a critical role in organising coherent signal transducing complexes that are essential for the appropriate cellular response to extracellular stimuli.

Constituitively active signal transduction pathways have been identified in a variety of disease states. Ligands that are able to disrupt these inappropriately hyperstimulated pathways, by blocking SH2 domain-dependant interactions, may ultimately find utility as therapeutic targets. Ligands directed against the Lck SH2 domain could serve in various capacities, such as for the treatment of autoimmune diseases and T cell-based leukemias and lymphomas.

A combinatorial chemistry approach has been used to determine which residues of the tetrapeptide peptide ligand (i) were critical for binding to the SH2 domain (SH2-Directed ligands of the Lck tyrosine kinase, T. R. Lee and D. S. Lawrence, *J. Med. Chem*., 43, (2000), 1173-1179). One library of 84 individual compounds was synthesised on Tentagel S NH_2 resin and was used to determine whether the Glu-Glu residue of (i) were essential for binding to the SH2 domain. Learning was incorporated into the synthesis of a second library of 900 individual compounds, also synthesised on Tentagel S NH_2 resin, for the purpose of acquiring non-amino acid mimetics for the P+4 Ile moiety. One of the most potent compounds prepared from this library was (ii), which possessed an IC_{50} of 660 nM. This work has demonstrated that three amino acid residues of the SH2 ligand (i) can be replaced with non-amino acid substituents without loss of affinity. Future studies could utilise this knowledge in the design of higher affinity ligands for SH2 domains than that displayed by conventional peptide ligands, thereby providing potential treatments for a variety of medical disorders.

Ac-pTyr-Glu-Glu-Ile-NH2

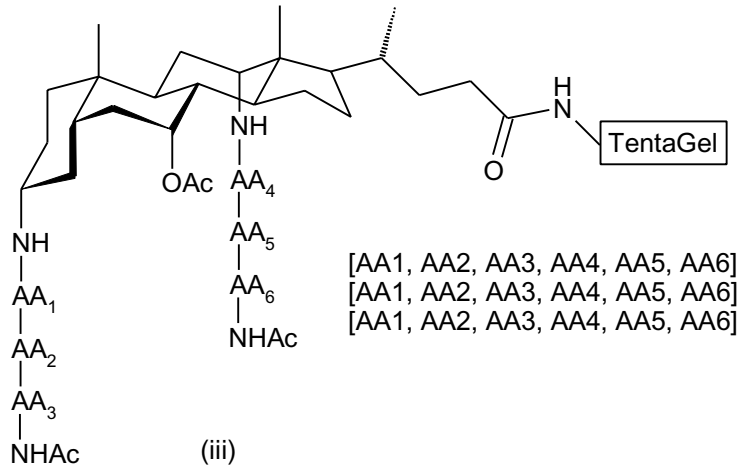
(i)



(ii)

Serine protease-like activity

The cleavage of peptide bonds by the serine protease α-chymotrypsin, for example, is a highly efficient and selective reaction compared to its uncatalysed hydrolysis. Although progress has been made in development of synthetic hydrolases, substantial improvement is still needed. One area of research has been the development of synthetic hydrolases with the focus on gaining a better understanding of the enzymatic process itself. In particular, development of non-peptidic organic molecules possessing an array of functional groups in a suitable geometry for eventual activity. A study in which serine protease activity is searched by combinatorial techniques in which the two most important catalytic residues, Ser and His of the classic triad (Ser, His, Asp), are each incorporated into one of the two tripeptidic chains generated on a steroidal scaffold (iii) has been undertaken (Application of combinatorial procedures in the search for serine-protease-like activity with focus on the acyl transfer step, P. J. De Clercq *et. al., Angew. Chem. Int. Ed*., 39, (2000), 145-148). A library of 729 compounds in pools of 234 members, prepared using the split-mix synthesis procedure on TentaGelNH_2 solid phase resin, revealed three potent mixtures on initial screening against an activated para-nitrophenol ester, which was used as a model for the first step of the enzymatic mechanism. Recursive deconvolution identified (iii a-c) as the most potent constituents of these mixtures. This work may aid the future design of synthetic hydrolases which could lead to a better understanding of the mechanism of cleavage and, ultimately, to the synthesis of medicinally relevant compounds.



(iii)

[AA1, AA2, AA3, AA4, AA5, AA6] = [Gly, Phe, Ser, Leu, His, Ala] (iii a)
[AA1, AA2, AA3, AA4, AA5, AA6] = [Phe, Ser, Phe, Leu, His, Ala] (iii b)
[AA1, AA2, AA3, AA4, AA5, AA6] = [Ser, Ser, Phe, Leu, His, Ala] (iii c)